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Effects of α-Glycerophosphate and of Palmityl-Coenzyme A on Lipid Synthesis in Yeast Extracts

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ABSTRACT

WHITE, DAVID (Ames Research Center, Moffett Field, Calif.), AND HAROLD P. KLEIN. Effects of α-glycerophosphate and of palmityl-coenzyme A on lipid synthesis in yeast extracts. J. Bacteriol. **91**:1218–1223. 1966.—The incorporation of acetate into fatty acids, but not into nonsaponifiable lipids, was stimulated by α-glycerophosphate in a supernatant fraction of *Saccharomyces cerevisiae*, obtained after centrifugation at 86,000 × *g* for 60 min. There was a pronounced effect at concentrations below 2 mM, but at concentrations above 5 mM α-glycerophosphate was relatively less stimulatory. α-Glycerophosphate markedly increased the percentage of esterified fatty acids among the products, and the formation of both saturated and unsaturated fatty acids was stimulated. Palmityl-coenzyme A inhibited fatty acid synthesis, affecting the formation of unsaturated acids more severely than saturated acids. In the presence of sufficient α-glycerophosphate to alleviate these inhibitions, palmityl-coenzyme A still reduced the formation of certain unsaturated fatty acids.

In recent years, there have been several reports indicating that the rates of fatty acid synthesis in animal preparations are stimulated by various glycolytic intermediates (1, 4, 5, 12, 15), Krebs cycle intermediates (16, 18), and, in one instance, by α-glycerophosphate (6). The observed stimulations of fatty acid synthesis in the animal systems are the basis of current hypotheses concerning the physiological control of lipogenesis (6, 18). We recently reported that fatty acid synthesis in yeast extracts is increased by α-glycerophosphate or citrate (20). The present report is a continuation of those studies.

MATERIALS AND METHODS

Growth of organism and preparation of enzymes. Cells of *Saccharomyces cerevisiae* strain LK2G12 were grown as previously described and aerated for 2.5 hr in 10 times their packed volume of 0.1 M potassium phosphate buffer (*p*H 7) containing 1% D-glucose (7). The cells were washed twice with cold distilled water and disrupted in a French pressure cell in phosphate buffer (*p*H 6.5) as previously described (8), after which the suspension was centrifuged at 3,300 × *g* for 10 min to sediment the whole cells and debris, then at 14,500 × *g* for 30 min to sediment the mitochondrial fraction (7), and finally at 86,000 × *g* for 60 min to sediment the ribosomal fraction (8) and yield a clear, high-speed supernatant (HSS). Approximately 30 ml of the HSS was dialyzed overnight in 4 liters of 0.1 M potassium phosphate buffer (*p*H 6.5) containing 0.5 mM reduced glutathione.

Assay of lipid synthesis. Lipid synthesis was followed by the incorporation of radioactive precursors. All incubations were done in air at 30 C. The incubation mixtures were saponified in alcoholic potassium hydroxide and extracted with petroleum ether as previously described (20). To analyze the lipid classes, total lipids were extracted from nonhydrolyzed incubation mixtures according to the method of Bligh and Dyer (2), and chromatographed as described below. A sample of the total lipids was hydrolyzed in alcoholic potassium hydroxide, and the fatty acids and nonsaponifiable lipids were separated by washing with dilute sodium hydroxide.

Separation of lipids into classes. Total lipids were chromatographed on silica gel G with a petroleum ether-diethyl ether-acetic acid (80:20:1) solvent system (10). A mixture of standard lipids was spotted and chromatographed on the same plate for identification. A sample of the hydrolyzed sample was also chromatographed next to the nonhydrolyzed lipid samples as a further means of identifying the glyceride spots. Lipids were detected by staining with iodine vapor. Radioactive lipids were detected by exposing the plates to X-ray film. The lipids were eluted from silica gel with methanol.

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Gas chromatography. Fatty acids were methylated with boron trifluoride according to the method of Metcalfe and Schmitz (11), and chromatographed on a diethyleneglycol succinate column by use of an Aerograph gas chromatograph, model A-90.

Radioactivity determinations. Radioactivity was determined with a Packard Tricarb liquid scintillation counter. The scintillation fluid was 1 liter of toluene, 1 liter of dioxane, 0.6 liter of 100% ethyl alcohol, 130 g of naphthalene, 13 g of diphenyloxazole, and 0.25 g of methylphenyloxazolylbenzene.

Chemicals. Palmityl-coenzyme A (palmityl-CoA) was synthesized according to the method of Seubert (14) and was assayed by the hydroxamate method of Kornberg and Pricer (9) for insoluble hydroxamates. Coenzymes and glycolytic intermediates were purchased from Sigma Chemical Co., St. Louis, Mo., and Calbiochem. Creatinine phosphate and creatine kinase were purchased from Calbiochem. DL-α-Glycerophosphate (disodium) grade X was purchased from Sigma Chemical Co. Acetate-*1*-C^{14} was purchased from New England Nuclear Co., Boston, Mass.

RESULTS

Effect of α-glycerophosphate. We previously reported (20) that the fatty acid synthesizing activity in the HSS was sensitive to the levels of α-glycerophosphate in the incubation mixture. Figure 1 illustrates the typical response of these preparations to α-glycerophosphate. There was always a sharp increase in the amount of acetate incorporation at α-glycerophosphate concentrations of the order of 1 to 2 mM. As the α-glycerophosphate level was raised, there followed a plateau region, and, at concentrations above about 5 mM, a slow increase in activity with increased α-glycerophosphate. Nonsaponifiable lipid synthesis was not affected at any concentrations tested.

We reported earlier that citrate also stimulated fatty acid synthesis without affecting nonsaponifiable lipid synthesis (20). It was of interest to compare the response of fatty acid synthesis to α-glycerophosphate with the response to citrate. In contrast to α-glycerophosphate, 1 to 2 mM citrate had relatively little effect on fatty acid synthesis (Fig. 2). As the citrate levels were raised, however, fatty acid synthesis increased as a linear function of the citrate concentration.

Effect of palmityl-CoA and α-glycerophosphate. Recently, Howard and Lowenstein (6) observed a stimulation by α-glycerophosphate of fatty acid synthesis in rat liver and lactating mammary gland extracts, and suggested that the stimulation may have been due to the removal of inhibitory amounts of long-chain acyl-CoA molecules via glyceride synthesis. This suggestion was prompted by several reports indicating that acyl-CoA inhibited fatty acid synthesis in rat and pigeon liver extracts (13, 17). It was therefore of interest

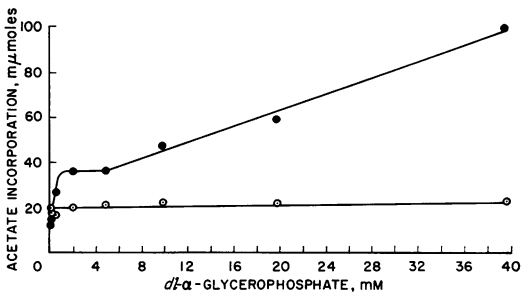

FIG. 1. *Effect of added α-glycerophosphate on lipid synthesis. The incubation mixtures contained the following: 10 mg of protein per ml, 1 mM acetate-1-C^{14} (1.2 × 10^6 counts per min per μmole), 2 mM adenosine triphosphate, 10 mM creatine phosphate, 0.26 mg/ml of creatine kinase, 2 mM $MnCl_2$, 2 mM reduced nicotinamide adenine dinucleotide, 0.05 mM CoA, 60 mM $KHCO_3$, and 70 mM potassium phosphate buffer (pH 6.5). Samples were incubated for 20 min in a total volume of 1 ml. Closed circles, fatty acids; open circles, nonsaponifiable lipids.*

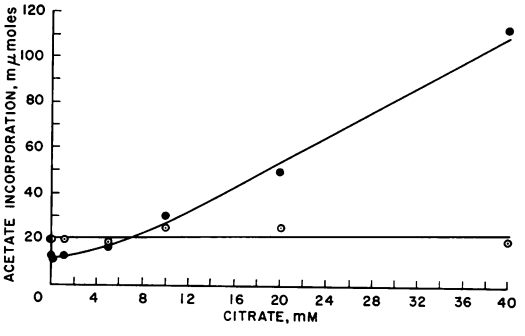

FIG. 2. *Effect of added citrate on lipid synthesis. See Fig. 1 for conditions. Closed circles, fatty acids; open circles, nonsaponifiable lipids.*

to determine whether acyl-CoA molecules were inhibitory also in the yeast preparation and, if so, whether α-glycerophosphate was capable of preventing the inhibition.

Figure 3 illustrates the inhibitory effect of increasing amounts of palmityl-CoA on fatty acid synthesis. (Nonsaponifiable lipid synthesis was not affected by these concentrations of palmityl-CoA). Note that the amount of palmityl-CoA required for 50% inhibition was approximately 3.2×10^{-5} M.

Table 1 shows that 7.5×10^{-5} M palmityl-CoA did not inhibit fatty acid synthesis when α-glycerophosphate was present. However, α-glycerophosphate did not protect fully against inhibition by the higher concentration of palmityl-CoA, explaining perhaps why we previously failed to

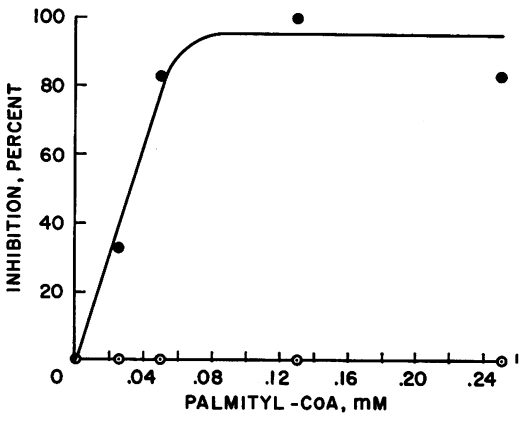

FIG. 3. *Effect of added palmityl-CoA on lipid synthesis. See Fig. 1 for conditions. Closed circles, fatty acids; open circles, nonsaponifiable lipids.*

observe a protective effect by α-glycerophosphate (20).

We also examined the effect of citrate on inhibition by palmityl-CoA. Table 2 shows that citrate, like α-glycerophosphate, protected fatty acid synthesis from the inhibitory effects of palmityl-CoA.

Effect of α-glycerophosphate on classes of lipids synthesized. If α-glycerophosphate does stimulate fatty acid synthesis by reacting with acyl-CoA to form glycerides, then an increase in esterified fatty acids should result when fatty acids are synthesized in the presence of α-glycerophosphate. Accordingly, an experiment was performed in which the lipid classes were examined after incubation in the absence and in the presence of α-glycerophosphate. Before chromatographing the total lipid fraction, a sample of each incubation mixture was saponified and separated into fatty acids and nonsaponifiable lipids. As is seen in Table 3, α-glycerophosphate stimulated fatty acid synthesis approximately eightfold in this experiment. And, as is evident from Table 4, the nonhydrolyzed samples showed a shift away from free fatty acids toward esterified fatty acids in the presence of α-glycerophosphate.

Effect of palmityl-CoA and α-glycerophosphate on types of fatty acids synthesized. As a further guide to the interpretation of the stimulation by α-glycerophosphate, the distribution of newly formed fatty acids was examined. Table 5 indicates that the synthesis of each long-chain fatty acid was significantly stimulated by α-glycerophosphate. On the other hand, palmityl-CoA did not inhibit the synthesis of each fatty acid to an equal extent. This effect was most striking in those samples which were incubated with α-glycerophosphate. In the latter samples, palmityl-CoA inhibited only the formation of C_{18} unsaturated fatty acids and a C_{14} unsaturated fatty acid (probably myristoleic), while actually stimulating the synthesis of shorter chain saturated fatty acids. The net result was a shift to saturated fatty acids of slightly lower chain lengths than those of the controls. It should be noted that in this experiment α-glycerophosphate completely prevented any net inhibition of fatty acid synthesis by palmityl-CoA.

TABLE 1. *Effect of palmityl-CoA and α-glycerophosphate on lipid synthesis**

Addition	Acetate incorporation (mμmoles)	
	Fatty acids	Nonsaponifiables
None	20	31
Palmityl-CoA (0.075 mM)	8	33
Palmityl-CoA (0.15 mM)	1	34
α-Glycerophosphate (10 mM)	68	30
α-Glycerophosphate (10 mM) + palmityl-CoA (0.075 mM)	74	37
α-Glycerophosphate (10 mM) + palmityl-CoA (0.15 mM)	39	35

* The incubation mixtures contained the following: 9 mg of protein per ml, 2 mM acetate-*1*-C^{14} (1.2×10^6 counts per min per μmole), 1.8 mM adenosine triphosphate, 9 mM creatine phosphate, 0.2 mg/ml of creatine kinase, 1.8 mM $MnCl_2$, 1.8 mM reduced nicotinamide adenine dinucleotide, 0.04 mM CoA, 60 mM $KHCO_3$, and 70 mM potassium phosphate (*p*H 6.5). The samples were incubated for 20 min in a total volume of 1 ml.

TABLE 2. *Effects of α-glycerophosphate and citrate in the presence and absence of palmityl-CoA**

Addition	Palmityl-CoA (0.075 mM)	Acetate incorporation (mμmoles)	
		Fatty acids	Nonsaponifiables
None	—	21	27
	+	7	24
α-Glycerophosphate (10 mM)	—	67	30
	+	72	32
Citrate (50 mM)	—	144	18
	+	109	26

* The incubation mixtures were similar to those cited in Table 1. Samples were incubated in a total volume of 1 ml for 20 min.

TABLE 3. *Influence of α-glycerophosphate on lipid synthesis**

Addition	Acetate incorporation (mμmoles)	
	Fatty acids	Nonsaponifiables
None	46	156
α-Glycerophosphate, 10 mM	327	158

* The incubation mixtures contained the following: 10 mg of protein per ml, 2 mM acetate-*1*-C^{14} (1.2×10^6 counts per min per μmole), 2 mM adenosine triphosphate, 10 mM creatine phosphate, 0.26 mg/ml of creatine kinase, 2 mM $MnCl_2$, 2 mM reduced nicotinamide adenine dinucleotide, 0.05 mM CoA, 60 mM $KHCO_3$, and 70 mM potassium phosphate buffer (*p*H 6.5). The samples were incubated for 75 min in a total volume of 5 ml. The total lipids were extracted and divided into two equal portions. One portion was hydrolyzed and the counts in fatty acids and nonsaponifiable lipids were determined. The results are reported as that amount of acetate incorporated in the original incubation mixture. The nonhydrolyzed portion was chromatographed on silica gel and the results recorded in Table 4.

TABLE 4. *Influence of α-glycerophosphate on the classes of lipids formed**

Lipid class	Per cent of radioactivity incorporated into fatty acid-containing materials	
	Without α-glycerophosphate	With α-glycerophosphate
Phospholipids†	11.3	24.8
Monoglycerides	22.9	60.4
Diglycerides	2.1	1.1
Free fatty acids	59.9	7.9
Triglycerides	3.7	5.7

* See Table 3 for experimental details.

† The phospholipid fraction contained a small amount of radioactivity which, after alkaline hydrolysis, behaved like a relatively nonpolar, nonsaponifiable lipid. No correction was made for these nonphospholipid counts.

DISCUSSION

Tubbs and Garland (17) and Bortz and Lynen (3) pointed out that negative feedback mechanisms may operate in fatty acid synthesis in animal cells. The experimental basis for this hypothesis began with a report by Porter and Long (13) that palmityl-CoA inhibited fatty acid synthesis in pigeon liver homogenates. More recent reports by Tubbs and Garland (17) and Bortz and Lynen (3) indicated that palmityl-CoA

TABLE 5. *Influence of α-glycerophosphate and palmityl-CoA on the individual fatty acids formed**

Fatty acid chain length†	Control	Palmityl-CoA (0.075 mM)	α-Glycerophosphate (10 mM)	α-Glycerophosphate + palmityl-CoA
12	820	970	1,640	3,480
12:0	970	930	2,790	10,180
12 to 14 (12:1?)	0	90	0	0
14:0	1,580	1,310	5,580	17,770
14 to 16 (14:1?)	240	130	14,760	1,790
16:0	3,720	1,280	17,640	29,210
16:1	2,570	370	13,530	14,200
16 to 18 (16:2?)	350	95	2,050	1,970
18:0	970	570	3,720	2,860
18:1	8,850	2,270	15,500	5,360
>18:1	3,460	900	4,760	1,970
Total	23,530	8,915	81,970	88,790

* The experimental conditions were the same as those cited in Table 1. Results are expressed as acetate incorporation (counts per minute). More than 80% of the injected counts were recovered on each of these columns.

† Based on retention time of known standards; number of carbon atoms:number of double bonds.

inhibited, respectively, pigeon liver and rat liver acetyl-CoA carboxylation, the first reaction in the incorporation of acetyl-CoA into fatty acids. The low concentrations of palmityl-CoA required (3) to inhibit purified rat liver acetyl-CoA carboxylase ($K_I = 7.2 \times 10^{-6}$ M) and the competitive nature of the inhibition (competitive with respect to acetyl-CoA) argue for its physiological significance.

The inhibition of fatty acid synthesis by palmityl-CoA, as well as the stimulations by citrate and α-glycerophosphate, which are reported here, are probably at the site of acetyl-CoA carboxylation. This contention is based on several factors. First, it was found that acetate and acetyl-CoA incorporation into fatty acids was at least six times slower than that of malonyl-CoA (White, Ph.D. Thesis, Brandeis Univ., Waltham, Mass., 1965). In addition, compounds which stimulate fatty acid synthesis (including citrate and α-glycerophosphate) were shown also to stimulate acetyl-CoA carboxylation (20). Finally, palmityl-CoA, at concentrations which completely inhibited acetate incorporation into fatty acids, was far less effective when malonyl-CoA was the substrate (20).

The stimulation of animal acetyl-CoA car-

boxylase by citrate is well established (18). Vagelos, Alberts, and Martin (19) reported that citrate activation of rat adipose tissue acetyl-CoA carboxylase involved an apparent aggregation of the enzyme. The activated enzyme had a Svedberg constant of 43*S* as compared with 18.8*S* for the nonactivated enzyme. Although we have no evidence concerning the mechanism of activation of carboxylase in yeast, it is possible that the yeast carboxylase may exist in crude homogenates as a mixture of an activated high molecular weight enzyme, and a less active, smaller species. If this were true, then the HSS may be enriched with the smaller species. The HSS, therefore, may be a good source for an enzyme which can be activated by various modifiers.

The prevention of palmityl-CoA inhibition by addition of α-glycerophosphate is interesting. It would appear that even when the α-glycerophosphate was in 100-fold excess over the palmityl-CoA, not all the palmityl-CoA was removed by the α-glycerophosphate. This conclusion can be drawn from the decreases in the synthesis of unsaturated fatty acids and the reciprocal increases in saturated fatty acids when palmityl-CoA was present in incubation mixtures containing α-glycerophosphate. Indeed, the kinds of fatty acids synthesized may be more sensitive to palmityl-CoA than is overall fatty acid synthesis.

Since α-glycerophosphate was able to prevent the net inhibition of fatty acid synthesis by added palmityl-CoA, one might argue that the dialyzed HSS used here contained inhibitory amounts of endogenous acyl-CoA. The fact that α-glycerophosphate shifted the products from free fatty acids to glycerides implies the existence of a mechanism for the α-glycerophosphate-dependent removal of acyl-CoA.

Although the data presented are consistent with the notion that α-glycerophosphate stimulates fatty acid synthesis by removing acyl-CoA, they do not exclude another role for α-glycerophosphate. It should be noted that citrate, which probably is not a precursor for glyceride synthesis, also "reversed" inhibition by palmityl-CoA. In addition, α-glycerophosphate seemed to stimulate the synthesis of those fatty acids that were least affected by palmityl-CoA (Table 5).

A dual role for α-glycerophosphate is suggested by the response of fatty acid synthesis to increasing concentrations of α-glycerophosphate. Fatty acid synthesis increased sharply with low concentrations of α-glycerophosphate (of the order of 1 mM) and, above approximately 5 mM α-glycerophosphate, fatty acid synthesis slowly increased as a linear function of the α-glycerophosphate concentration. It is pertinent to note that the response of the yeast system at the higher concentration range resembled that of the citrate-activated systems (Fig. 1 and 2). Further studies may demonstrate that, at low concentrations, α-glycerophosphate stimulates by removal of acyl-CoA and at high concentration, by an allosteric modification of a part of the enzyme system.

LITERATURE CITED

1. ABRAHAM, S., AND I. L. CHAIKOFF. 1959. Glycolytic pathways and lipogenesis in mammary glands of lactating and non-lactating normal rats. J. Biol. Chem. **234**:2246–2253.
2. BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
3. BORTZ, W. M., AND F. LYNEN. 1963. The inhibition of acetyl-CoA carboxylase by long-chain acyl-CoA derivatives. Biochem. Z. **337**:505–509.
4. GIBSON, D. M., M. I. JACOB, J. W. PORTER, A. TIETZ, AND S. J. WAKIL. 1957. Biosynthesis of fatty acids by soluble enzyme fractions. Biochim. Biophys. Acta **23**:219–220.
5. HIRSCH, P. F., H. BARUCH, AND I. L. CHAIKOFF. 1954. The relation of glucose oxidation to lipogenesis in mammary tissue. J. Biol. Chem. **210**: 785–797.
6. HOWARD, C. F., AND J. M. LOWENSTEIN. 1964. The effect of α-glycerophosphate on the microsomal stimulation of fatty acid synthesis. Biochim. Biophys. Acta **84**:226–228.
7. KLEIN, H. P. 1957. Some observations on a cell free lipid synthesizing system from *Saccharomyces cerevisiae*. J. Bacteriol. **73**:530–537.
8. KLEIN, H. P. 1963. Stimulation of lipid synthesis by yeast ribosomal preparations. Biochim. Biophys. Acta **70**:606–608.
9. KORNBERG, A., AND W. E. PRICER, JR. 1953. Enzymatic synthesis of the coenzyme-A derivatives of long-chain fatty acids. J. Biol. Chem. **204**:329–343.
10. MANGOLD, H. K. 1961. Thin-layer chromatography of lipids. J. Am. Oil Chemists Soc. **38**: 708–727.
11. METCALFE, L. D., AND A. A. SCHMITZ. 1961. The rapid preparation of fatty-acid esters for gas chromatographic analysis. Anal. Chem. **33**:363.
12. PERRY, W. F. 1958. Acetate utilization by liver and adipose tissue of rats fasted in the cold. Can. J. Biochem. Physiol. **36**:237–241.
13. PORTER, J. W., AND R. W. LONG. 1958. A study of the role of palmityl-CoA in fatty acid synthesis by the pigeon liver system. J. Biol. Chem. **233**:20–25.
14. SEUBERT, W. 1960. S-palmityl coenzyme-A. Biochem. Prepn. **7**:80–83.
15. SHAW, W. N., F. DITURI, AND S. GURIN 1957 Lipogenesis in particle free extracts of rat liver J. Biol. Chem. **226**:417–425.
16. STUMPF, P. K. 1960. Lipid metabolism. Ann. Rev. Biochem. **29**:261–294.
17. TUBBS, P. K., AND P. B. GARLAND. 1963. Fatty

acyl thio esters of CoASH: inhibition of fatty acid synthesis *in-vitro* and determination of levels in liver in normal, fasted, and fat- or sugar fed rats. Biochem. J. **89**:25.

18. VAGELOS, P. R. 1964. Lipid metabolism. Ann. Rev. Biochem. **33**:139–172.
19. VAGELOS, P. R., A. W. ALBERTS, AND D. B. MARTIN. 1963. Studies on the mechanism of activation of acetyl-coenzyme-A carboxylase by citrate. J. Biol. Chem. **238**:533–540.
20. WHITE, D., AND H. P. KLEIN. 1965. Factors affecting fatty acid synthesis in cell-free preparations from *Saccharomyces cerevisiae*. Biochim. Biophys. Res. Commun. **20**:78–84.